

## APPLIED ISSUES

# The effects of macroinvertebrate taxonomic resolution in large landscape bioassessments: an example from the Mid-Atlantic Highlands, U.S.A.

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## SUMMARY

1. During late spring 1993–1995, the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) sampled 490 wadeable streams in the mid-Atlantic Highlands (MAH) of the U.S. for a variety of physical, chemical and biological indicators of environmental condition. We used the resulting data set to evaluate the importance of differing levels of macroinvertebrate taxonomic resolution in bioassessments by comparing the ability of family versus genus to detect differences among sites classified by type and magnitude of human impact and by stream size. We divided the MAH into two physiographic regions: the Appalachian Plateau where mine drainage (MD) and acidic deposition are major stressors, and the Ridge and Valley where nutrient enrichment is a major stressor. Stream sites were classified into three or four impact classes based on water chemistry and habitat. We used stream order (first to third Strahler order) in each region as a measure of stream size. Ordination,  $2 \times 2$  chi-square and biotic metrics were used to compare the ability of family and genus to detect differences among both stressor and size classes.
2. With one notable exception, there were only a small number of different genera per family (interquartile range = 1–4). Family Chironomidae, however, contained 123 different genera. As a result, significant information loss occurred when this group was only classified to family. The family Chironomidae did not discriminate among the predefined classes but many chironomid genera did: by chi-square analysis, 10 and 28 chironomid genera were significant in discriminating MD and nutrient impacts, respectively.
3. Family and genus data were similar in their ability to distinguish among the coarse impacts (e.g. most severe versus least severe impact classes) for all cases. Though genus data in many cases distinguished the subtler differences (e.g. mixed/moderate impacts versus high or low impacts) better than family, differences in significance levels between family and genus analyses were relatively minor. However, genus data detected differences among stream orders in ordination analyses that were not revealed at the

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family level. In the ordinations, both family and genus levels of analysis responded to similar suites of environmental variables.

4. Our results suggest that identification to the family level is sufficient for many bioassessment purposes. However, identifications to genus do provide more information in genera-rich families like Chironomidae. Genus or finer levels of identification are important for investigating natural history, stream ecology, biodiversity and indicator species. Decisions about the taxonomic level of identification need to be study specific and depend on available resources (cost) and study objectives.

*Keywords:* bioassessment, macroinvertebrates, Mid-Atlantic, multivariate, richness, streams, taxonomic resolution

## Introduction

The use of macroinvertebrates in stream bioassessments has increased markedly in the past 20 years (Cairns & Pratt, 1993 and Lenat & Resh, 2001), yet there is still much debate as to the taxonomic identification level and sample size necessary to determine anthropogenic impacts (Resh & McElravy, 1993). An important biomonitoring question is: does finer taxonomic resolution of macroinvertebrates (for example classification to genus or species versus family) allow better discrimination among sites differentially impacted by human activity than higher taxonomic levels? The obvious answer is that it is contextual (Bailey, Norris & Reynoldson, 2001; Lenat & Resh, 2001). Resh & McElravy (1993) summarise this point well: 'In examining the importance of species level identifications in biomonitoring, the question of the required level of taxonomy still can be asked. The answer, it seems, is "it depends" – it depends on the purpose of the study, the level of sensitivity required, the type of index or analysis being used, and the particular group of organisms of primary interest.' Clearly, if differences among sites or classes of sites are great, a coarser taxonomic resolution is likely to suffice for detecting differences; if differences among sites/classes are small it is possible that finer taxonomic resolution is likely necessary to detect differences. Lenat & Resh (2001) provide a list of recommendations for appropriateness of family or genus/species taxonomy: family is appropriate when the goal is to determine relatively large between-site differences; genus/species is required when small between-site or between-date differences must be detected, such as when separating sites into more than three water quality categories.

In general, the results in the literature suggest that classification to family is sufficient to detect impacts

from point sources and other coarse impacts in both freshwater and marine systems (Ferraro & Cole, 1995; Wright *et al.*, 1995; Vanderklift, Ward & Jacoby, 1996), but what about other types of impacts and gradients? Bowman & Bailey (1997) reviewed 10 freshwater studies and found that genus performed better than family or order when using presence-absence, yet for abundance, genus provided the same description of community patterns as family or order. Although species had higher predictive success in stream site classification than family, Furse *et al.* (1984) suggest that family was sufficient to detect the important environmental gradients related to the sites. They also suggested that identification to family could offer a substantial time and cost savings over identification to species. Marchant, Barmuta & Chessman (1995) found that the community pattern among 40 stream sites along an altitudinal gradient was preserved when quantitative species data were converted to quantitative or qualitative family data. The same pattern was also found using just EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa at the family level. Marchant *et al.* (1995) suggested that the level of identification necessary to determine an environmental gradient depends upon the spatial scale of the investigation and that family should be sufficient over a wide geographic area. Hewlett (2000) assessing reference sites across a large geographic area of Southeastern Australia also found that family and EPT species were as good as full-species data in classifying streams into groups. Reynoldson, Rosenberg & Resh (2001) developed classification models of 219 sites in the Fraser River basin and found that family data had a better classification rate than either genus or species data with rare taxa removed. These studies, however, were all completed across landscape

gradients that were not significantly affected by non-point source pollution.

Most of these studies show that when there are marked differences among sites because of clear impacts such as sewage effluent or there is a large environmental gradient (e.g. wide geographic area with large ecoregion changes) such as altitude, family level taxonomic resolution is sufficient to differentiate among sites. However, will family be sufficient when the effects or gradients are subtler, such as low-level non-point source pollution? Vanderklift *et al.* (1996) state: 'Different assemblages collected along different gradients may yield other results. For example, assemblages along weaker natural gradients may not yield robust patterns like those seen along a strong pollution or habitat gradient.' In contrast to the coarse impacts studied by Vanderklift *et al.* (1996) and Ferraro & Cole (1995), Hawkins *et al.* (2000) found that species based models were able to detect subtle impacts in streams in California because of non-point source effects of logging that family based models were not able to detect. Macroinvertebrate bioassessments are often used to discern a wide variety of natural and anthropogenic gradients within and among ecoregions. Therefore, the effects of taxonomic resolution should be assessed across a variety of gradients and biomes/ecoregions.

To address the general question of additional information gained by classifying to finer levels of taxonomy, we compared the ability of family versus genus to distinguish among several independently defined classes of wadeable stream sites using data from 490 sites in the Mid-Atlantic Highlands (MAH) in the U.S.A. We divided the MAH into two regions, the Appalachian Plateau, in which major impacts are mine drainage and acidic deposition considered to be coarse impacts (direct toxicity and habitat impacts), and the Ridge and Valley, in which major impacts are from more subtle non-point source pollution (i.e. high nutrient loadings, no direct toxicity). This large data set gave us the unique ability to test taxonomic resolution across a large geographic region affected by the two different perturbation types and thus address the coarse versus subtle distinction considered appropriate for family versus genus data. We also used stream order (Strahler) as a measure of stream size as another classification model against which to compare the two taxonomic levels.

## Methods

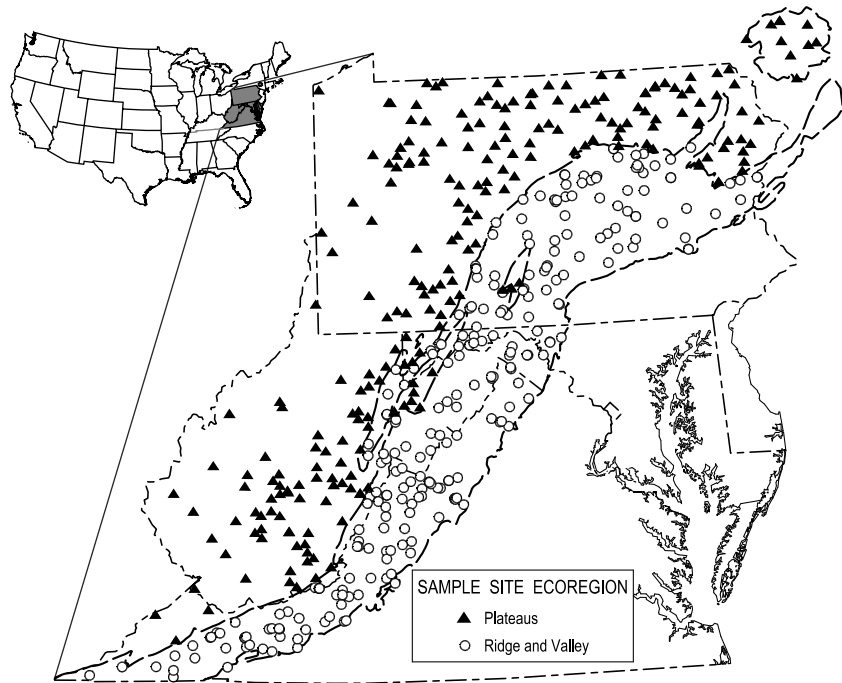
### Study region

The U.S. Environmental Protection Agency (USEPA) conducted a monitoring study of the biological condition of streams in the MAH covering parts of the states of Pennsylvania, Maryland, Virginia, West Virginia and New York as part of the Environmental Monitoring and Assessment Program (EMAP). The USEPA MAH monitoring study has been described in detail (USEPA, 2000; Waite *et al.*, 2000, and Herlihy *et al.*, 2000), and we will only provide a brief overview. The MAH is a physiographically and geologically diverse region consisting of a series of ridges and valleys, running roughly along a NE-SW axis, in the east, and sharply dissected plateaus to the west (Fig. 1). Omernik (1994) and Woods *et al.* (1996) delineated and described the ecoregions of this area (Blue Ridge Mountains, Ridge and Valley, Central Appalachians and the North Central Appalachians). In general, there can be extensive differences within and among ecoregions in parent geology. The MAH also has a large latitudinal extension, and thus, southern areas may be warmer than the northern regions.

We focus our analysis on two sub-regions of the MAH, the Plateaus (Central, North-Central and Northern Appalachian Plateau and Western Appalachians ecoregions) and the Ridge and Valley ecoregion (Fig. 1). Major disturbances in the Plateau region are mine drainage and acidic deposition, while the predominant impacts in the Ridge and Valley are non-point source discharges of nutrients and sediment from agriculture.

### Development of the data sets

Data for this study were compiled from the EMAP surveys conducted in the MAH in 1993–95 (Fig. 1). Each year, a regionally representative set of sample sites was selected from the digital version of 1 : 100 000 scale U.S. Geological Survey hydrologic maps using a randomised sampling design with a systematic spatial component (Herlihy *et al.*, 2000). The survey was restricted to wadeable streams (first to third Strahler order) as delineated on 1 : 100 000 scale digital maps. Streams were sampled in the spring (primarily May and June) for a suite of biological, chemical and physical habitat attributes (Lazorchak,



**Fig. 1** Location of stream sampling sites in the Mid-Atlantic Highlands (MAH) study area for the Plateau ( $n = 239$ ) and Ridge and Valley ( $n = 251$ ) regions.

Klemm & Peck, 1998). In addition, 68 handpicked sites were sampled to evaluate reference (58 minimally impacted) and test sites (10).

At each site, the study reach consisted of a stream length equal to 40 times the mean wetted width (150 m minimum). Macroinvertebrates were sampled at nine equidistant transects at each site using a modified kick net (595  $\mu\text{m}$  mesh; Lazorchak *et al.*, 1998). Riffle or pool transect samples were placed into separate buckets and composited. Transects were considered to be 'riffle' if there was measurable flow at the point of collection and 'pool' if there was no noticeable flow. Samples were preserved in 95% ethanol. In the laboratory, each composite sample was enumerated to the lowest practical taxonomic level (commonly genus or higher, Table 1) using a

300-organism fixed count protocol (Klemm & Lazorchak, 1994).

A 4-L cubitainer and four, 60-mL syringes of stream water were collected in flowing water near the middle of each selected stream reach. Water samples were placed on ice and sent by overnight courier to the analytical laboratory for measurement of Fe, Mn,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ , dissolved organic carbon (DOC), turbidity, total suspended solids (TSS), dissolved Si and  $\text{NH}_4^+$ , conductivity, pH, Gran acid neutralising capacity (ANC) and total nitrogen (TN) and phosphorus (TP) (USEPA, 1987). Based on multiple measurements of audit samples, analytical precision was  $\pm 5\%$  or less (coefficient of variation) or  $\pm 5 \mu\text{eqL}^{-1}$  (standard deviation) for all analyses. Detailed description of water chemistry collection and laboratory analysis along

**Table 1** Per cent of specimens and number ( $N$ ) of taxa identified to family, genus and species by macroinvertebrate group for both Mid-Atlantic Highlands regions combined (Ave = weighted average)

Taxa Group	Total specimens	Family		Genus		Species	
		(%)	$N$	(%)	$N$	(%)	$N$
Ephemeroptera	33 886	98	14	77	32	25	57
Plecoptera	19 144	98	9	85	38	1	14
Trichoptera	9380	99	18	90	50	17	24
Diptera	48 171	100	18	83	172	29	144
Other insect orders	11 013	100	29	95	61	38	34
Non-insects	8874	97	30	76	60	43	65
Total	130 468	Ave 99	118	Ave 83	413	Ave 25	338

with comparison with land use is provided in Herlihy, Stoddard & Johnson (1998).

The USEPA rapid bioassessment protocols (RBP) were used to make an overall physical habitat assessment for each site (Barbour & Stribling, 1991). The RBP habitat protocol involved rating 12 aspects of physical habitat on a scale from one to 20 and then summing the 12 scores together for an overall rating (maximum = 240 points). For each sample site, site altitude and catchment slope were determined from the altitude contours on 1 : 24 000 scale USGS maps. Catchment slope was calculated as the difference in altitude between the sample site and the highest point in the catchment divided by the straight-line distance between them. Site ecoregion locations were based on the ecoregion boundaries depicted in the MAH ecoregion delineations found in Woods *et al.* (1996).

#### *Data analysis*

We trimmed the full dataset in the following ways: only riffle samples were used because pool samples were collected at approximately half the sites, whereas riffle samples were collected at over 90% of the sites. We restricted our comparisons with family versus genus level data because an insufficient number of individuals (25%; Table 1) were classified to species.

*Stream classification.* We used several methods to evaluate the ability of family or genus to distinguish classes of stream sites. In the Plateau, we identified four stream classes based on stream chemistry: acidic deposition affected (AD, high impact or coarse), mine drainage affected (MD, high impact), mixed and low impact. Sample sites were divided into these four classes using a scheme similar to that used in MAH by Herlihy *et al.* (1990) and Herlihy, Kaufmann & Mitch (1991): (i) AD impacts –  $\text{ANC} < 25 \mu\text{eq L}^{-1}$  and sulphate  $< 400 \mu\text{eq L}^{-1}$ , (ii) MD impacts –  $\text{ANC} < 25 \mu\text{eq L}^{-1}$  and sulphate  $> 400 \mu\text{eq L}^{-1}$  or sulphate  $> 1000 \mu\text{eq L}^{-1}$ , (iii) Mixed impacts – all sites not classified into one of the other three classes (intermediate levels of MD and AD in addition to the influence of low levels of agricultural and urban land use) and (iv) Low impacts –  $\text{ANC} > 25 \mu\text{eq L}^{-1}$  and sulphate  $< 400 \mu\text{eq L}^{-1}$  and chloride  $< 100 \mu\text{eq L}^{-1}$ . All sites with an ANC below  $25 \mu\text{eq L}^{-1}$  were assumed to be acid

impacted and assigned to either the AD or MD impact class using sulphate concentration. Streams with ANC below  $25 \mu\text{eq L}^{-1}$  are either chronically acidic (no acid neutralising capacity;  $\text{ANC} < 0$ ) or usually transiently acidic ( $\text{ANC} 0\text{--}25$ ). In the Ridge and Valley, we broke stream sites into three classes based on nutrient concentrations: (i) high nutrient –  $\text{TN} > 1000 \mu\text{g L}^{-1}$  or  $\text{TP} > 30 \mu\text{g L}^{-1}$ , (ii) moderate – nutrient values between the high and low nutrient classes and (iii) low nutrient –  $\text{TN} < 300 \mu\text{g L}^{-1}$  and  $\text{TP} < 10 \mu\text{g L}^{-1}$ .

*Environmental gradients.* We also evaluated the consistency in response of family and genus to environmental gradients: did one taxonomic level detect gradients more clearly than the other? The similarity of sites based on abundance of macroinvertebrates can be visually portrayed on graphs that can demonstrate gradients such as detrended correspondence analysis (DCA; Jongman, ter Braak C.J.F. & Van Tongeren, 1995). DCA ordinations were run using the program PC-ORD (McCune, B. and M. J. Mefford, 1999. PC-ORD Multivariate Analysis of Ecological Data, Version 4, MjM Software Design, Gleneden Beach, OR, U.S.A.). Data were converted into relative abundance and rare taxa were down-weighted to reduce any unwarranted influence. All sites were kept in the ordinations. The first two family and genus axes scores were correlated with the measured water chemistry variables, a habitat index, altitude and catchment area using SAS (SAS/Stat user's guide, version 6.0, 4th edition, volume 2, SAS Institute, Cary, NC) and the significant environmental gradients compared. Family and genus ordinations were also compared with respect to their ability to distinguish the predefined chemical site classes by subjecting the axis 1 and axis 2 scores to a one-way analysis of variance; ANOVA was run on all sets of classes. Pairwise comparisons were completed using a Tukey's Studentised range test of differences in the means for each impact class. The significance statistic was corrected by the Bonferroni equation for all pair-wise tests. We also used the three stream orders as an approximation of three size classes against which to compare taxonomic resolution.

*Chi-square analysis.* For each taxon (family or genus), a two-way chi-square analysis using presence/absence was calculated to determine if the taxon

was able to distinguish between the highest and lowest impact classes by region. Only the sites in the low impact and most impacted classes (MD in the Plateau region and high nutrient in the Ridge and Valley region) were used in each regional dataset. For example, in the Ridge and Valley we analysed 142 sites (86 high nutrient, 56 low nutrient); the family Ameletidae was present at 25 sites (one high nutrient, 24 low nutrient) and absent from 117 sites (85 high nutrient, 32 low nutrient). The  $2 \times 2$  chi-square for this distribution was 40.6 and had a  $P < 0.001$  indicating that the distribution of Ameletidae across these sites was very likely because of the class effect and not random chance. The number and per cent of the families and genera that were able to detect a significant difference ( $P \leq 0.05$ ) between the reference and impacted sites were used as comparison metrics.

**Bioassessment metrics.** Taxa richness, EPT richness (Ephemeroptera, Plecoptera and Trichoptera), Hilsenhoff Biotic Index (HBI) and per cent dominance of the top five taxa were calculated for each site at family and genus. Box plots and pairwise comparisons of the means (Tukey's) were developed for comparison of richness measures between family and genus within and among impact classes for taxa and EPT richness metrics. For brevity, results from the HBI and per cent dominance metrics are presented within the text only.

## Results

### Characteristics of the dataset

In total, macroinvertebrate specimens were successfully identified to 29 orders, 128 families, 490 genera and 420 species. A great deal of effort was made to identify specimens to the lowest possible taxonomic level, however only 25% were able to be identified to species, 83% to genus, yet 99% to at least family (Table 1). The percentage of individuals identified to species varied among the major orders, for example 43% for non-insects but only 1% for Plecoptera. There were 86 families and 290 genera in the Plateau data set and 98 families and 319 genera in the Ridge and Valley data set.

Mean and standard deviations for water chemistry parameters and RBP habitat scores by impact class for acid mine drainage and acidic deposition (Plateau) and nutrient (Ridge and Valley) classification schemes are presented in Table 2.

### Relative discrimination power: family versus genus

From the Plateau and Ridge and Valley regions we considered AD, MD and high nutrients as 'coarse' impacts and mixed/moderate as a 'subtle' impact; by analogy, the comparison of first and third order streams was 'coarse' and first versus second and second versus third was 'subtle'. A synthesis of the results reveals the following patterns:

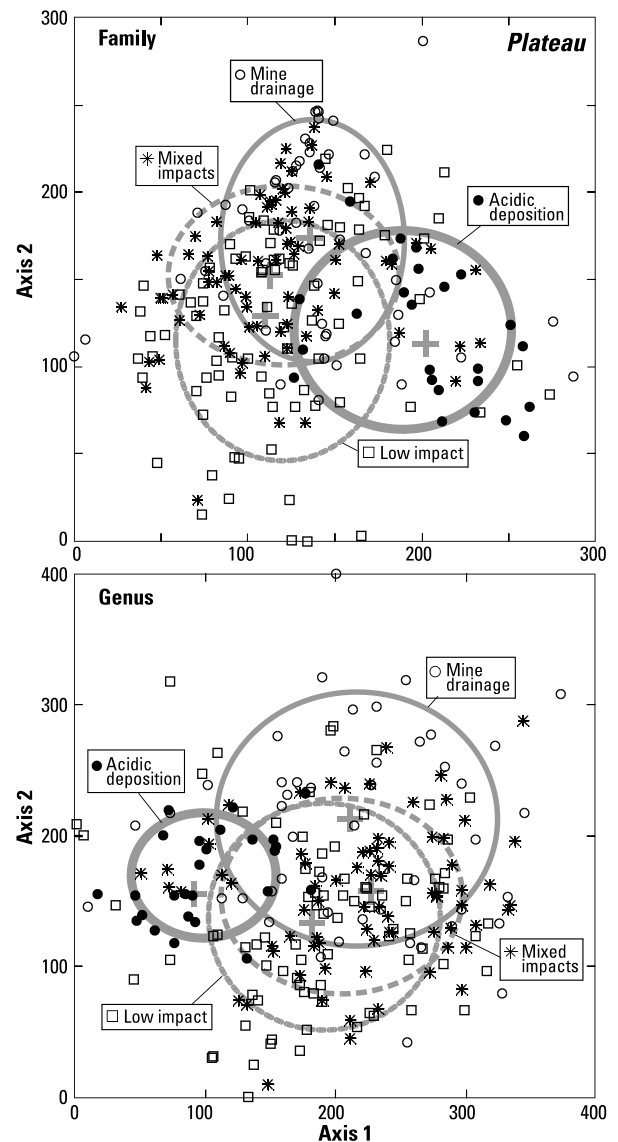
**Table 2** Mean and standard deviation (SD) of select parameters used to determine impact classes for stream sites from the Plateau and Ridge and Valley regions

Class	N	pH		Sulphate ( $\mu\text{eq L}^{-1}$ )		Chloride ( $\mu\text{eq L}^{-1}$ )		RBP habitat	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plateau									
Acid deposition	26	5.4	0.6	143	44	45	57	17	1.9
AMD impacts	52	6.7	1.5	3764	5220	215	318	14	2.8
Mixed impact	84	7.3	0.5	310	209	244	219	14	2.4
Least impact	77	7.1	0.5	200	65	40	23	15	2.6
	N	TN ( $\mu\text{g L}^{-1}$ )		TP ( $\mu\text{g L}^{-1}$ )		Chloride ( $\mu\text{eq L}^{-1}$ )		RBP habitat	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ridge & Valley									
High nutrient	86	2790	2347	65	111	296	365	13	2.9
Moderate	109	503	233	13	7	136	310	14	3.0
Low nutrient	56	141	66	6	2	49	86	15	2.2

N = number of sites.

*Coarse impacts: Plateau.* Overall for this region, genus was only slightly better than family in distinguishing among the coarse impacts for both AD/MD and stream order effects (e.g. AD and MD versus low impact). Ordination: both family and genus distinguished the coarse impacts with approximately equivalent resolution and showed similar significant differences although AD sites were separated better than MD sites from the other sites (Fig. 2; Table 3). Comparing first versus third order stream's ordination scores indicated similar significance at both family and genus. Analyses of individual taxa (chi-square) suggested that a greater proportion of families (28%,  $n = 24$  of 86) than genera (16%,  $n = 47$  of 290) discriminated the MD sites, but about twice the number of genera versus families discriminated (Table 4). For total and EPT richness there were slight differences in significance among the pair-wise comparisons between family and genus in the Plateau region (Fig. 3). Both family and genus total richness showed the primary differences among AD/MD and low impact sites, yet family EPT richness only detected differences in MD versus low impact, whereas genus EPT detected differences among both AD and MD versus low impact. Family and genus HBI and dominance of top five taxa detected the coarse impact class comparisons equally well with only minor differences.

*Coarse impacts: Ridge and Valley.* The genus ordination was slightly better at separating the high nutrient sites from the low nutrient sites (both axes significant) than was the family ordination (only first axis significant, Fig. 4), nonetheless, both were able to statistically distinguish the major nutrient gradient on the first axis (Table 3). Genus detected significant differences between first and third order sites on the first and third axes, but family was not able to detect any significant differences. As well, approximately the same proportion of families (21%,  $n = 21$  of 98) as genera (22%,  $n = 70$  of 319) distinguished the two groups of sites using chi-square, but three times the number of genera were significant compared with families. EPT richness at both family and genus were significantly different between the high and low nutrient sites (Fig. 5), but only family for total richness. HBI coarse comparison (high versus low nutrient) was significant for both family and genus, but per cent dominance was significant only for family. Therefore, for this region, the two taxonomic



**Fig. 2** The first two axes of the detrended correspondence analysis ordination plot of 239 sites at the family and genus level for the Plateau region, coded by impact class: acidic deposition, mine drainage, mixed impact and low impact. Ellipses are drawn for each impact class based on the 90th and 10th percentile of the axis scores; the centroid for each ellipse is designated by a large +. Three mine drainage sites ( $>400$  on either axis 1 or 2) are not shown on the genus plot so that the plot could be expanded for better visual clarity.

levels were similar in detecting the coarse impacts; however, genus was significantly better in distinguishing among stream size differences (Table 3).

*Subtle impacts: Plateau.* Both family and genus were similar in their ability to detect the more subtle impacts, although genus in general had higher levels

**Table 3** Results from one-way ANOVA comparison of impact classes and stream order related to the ordination axes (1 and 2) of family and genus level analyses for the Plateau and Ridge and Valley regions. Values are the mean axis score differences for each pair-wise comparison

Pair-wise comparisons	Family		Genus	
	Axis 1	Axis 2	Axis 1	Axis 2
Plateau				
F-value	24.1***	13.8***	20.1***	16.2***
Coarse				
AD – low	82.7***	–0.4	–87.8***	–33.1
MD – low	19.4	49.2***	26.4	77.8***
Subtle				
AD – MD	63.3***	–49.6***	–114.2***	–44.7**
AD – mixed	85.8***	–30.0*	–117.6***	13.6
MD – mixed	22.5	19.6	–3.4	58.3***
Mixed – low	–3.2	29.6*	29.8	19.5
F-value	19.2***	3.4*	51.8***	0.18
First order – third order (C)	49.0***	–21.4*	–105.0***	1.7
First order – second order (S)	26.3**	–9.0	–58.1***	–4.9
Second order – third order (S)	22.7*	–12.4	–47.0***	6.6
Ridge and Valley				
F-value	33.0***	0.82	52.6***	6.9**
High – low (C)	77.0***	4.2	131.8***	34.6***
High – moderate (S)	51.6***	6.4	89.9***	–6.7
Moderate – low (S)	25.4*	2.4	41.9**	–27.8*
F-value	1.4	0.04	4.8**	2.0
First order – third order (C)	–11.8	1.1	–42.2*	–18.3
First order – second order (S)	5.2	–0.3	–4.5	–8.8
Second order – third order (S)	–17.0	1.4	–37.7*	–9.5

Impact class abbreviations, Plateau: AD, acidic deposition; MD, mine drainage; mixed (intermediate) impacts and low impacts.

Ridge and Valley: classes refer to high, moderate and low nutrient conditions.

Statistical values are corrected for multiple comparisons. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; C, coarse; S, subtle comparisons.

of significance. Both family and genus were similar in distinguishing the subtle impact differences (e.g. AD-MD, AD-mixed, MD-mixed and mixed-low) in the ordinations (Table 3); yet, a more significant overlap of points encompassing mixed and the other classes is evident (Fig. 2). In general, genus detected some significant differences that family did not (e.g. MD versus mixed – ordination; AMD versus mixed – HBI; AD versus mixed – per cent dominance and total richness Fig. 3), but family detected some differences that genus did not (e.g. mixed versus low – ordination; AD versus mixed – HBI; mixed versus clean – per cent dominance; AD versus MD – total richness). When related to stream order, genus was slightly better at distinguishing first from second and second from third order with  $P < 0.001$  compared with family ( $P < 0.01$  and  $0.05$ , respectively).

*Subtle impacts: Ridge and Valley.* Genus seemed to detect the natural subtle impacts much better in this region than family, but was only slightly better related

to the anthropogenic impacts. Ordinations indicated significant overlap between moderate and low nutrient sites at both family and genus (Fig. 4). Significant differences between high-moderate and moderate-low comparisons could be detected with both family and genus (Table 3). Differences between first and second order (third axis  $P < 0.001$ ) and between second and third order streams (first axis) were detected with genus, but not with family data. Total and EPT richness, HBI and per cent dominance at both family and genus were significant for high versus moderate nutrient sites, though none of them were able to distinguish the moderate versus low comparisons (Fig. 5).

#### *Environmental and ecological patterns*

*Ordinations.* Ordination axis scores were significantly correlated with the same environmental variables for at least one axis for both taxonomic levels in both regions. Variables significantly correlated to at least one ordination axis for both taxonomic levels for



**Table 4** Total number of families and genera by insect order that had significant ( $P \leq 0.05$ ) chi-square scores for differences between low and high impact classes as well as the top 15 significant taxa for either the Plateau (mine drainage only) or Ridge and Valley data sets from the Mid-Atlantic Highlands. Total number of possible taxa given in parenthesis

Order	Plateau		Ridge and Valley	
	Family	Genus	Family	Genus
Ephemeroptera	5 (10)	13 (26)	5 (12)	10 (31)
Plecoptera	7 (8)	10 (30)	4 (9)	8 (28)
Trichoptera	5 (15)	5 (34)	3 (17)	7 (38)
Diptera	2 (14)	15 (134)	1 (13)	32 (136)
Other insects	4 (17)	4 (34)	2 (21)	6 (45)
Non-insects	0 (11)	0 (21)	6 (20)	7 (42)
Total	23 (75)	47 (279)	21 (92)	70 (320)
Significant taxa				
	Heptageniidae (E)	<i>Epeorus</i> (E)	Ameletidae (E)	<i>Ameletus</i> (E)
	Ephemerellidae (E)	<i>Drunella</i> (E)	Nemouridae (P)	<i>Lepidostoma</i> (T)
	Simuliidae (D)	<i>Baetis</i> (E)	Lepidostomatidae (T)	<i>Amphinemura</i> (P)
	Perlodidae (P)	<i>Ephemerella</i> (E)	Leuctridae (P)	<i>Epeorus</i> (E)
	Baetidae (E)	<i>Isoperla</i> (P)	Chloroperlidae (P)	<i>Leuctra</i> (P)
	Chloroperlidae (P)	<i>Oulimnius</i> (C)	Polycentropodidae (T)	<i>Prosimulium</i> (D)
	Philopotamidae (T)	<i>Stenonema</i> (E)	Perlodidae (P)	<i>Cryptochironomus</i> * (D)
	Pteronarcyidae (P)	<i>Hexatoma</i> (D)	Gammaridae (A)	<i>Constempellina</i> * (D)
	Sialidae (M)	<i>Micropectra</i> * (D)	Asellidae (I)	<i>Oulimnius</i> * (C)
	Tipulidae (D)	<i>Simulium</i> (D)	Tubificidae (Oligo)	<i>Optioserous</i> * (C)
	Rhyacophilidae (T)	<i>Sweltsa</i> (P)	Leptophlebiidae (E)	<i>Cinygmula</i> (E)
	Peltoperlidae (P)	<i>Pteronarcys</i> (P)	Simuliidae (D)	<i>Polycentropus</i> (D)
	Lepidostomatidae (T)	<i>Sialis</i> (M)	Naididae (Oligo)	<i>Gammarus</i> (A)
	Leuctridae (P)	<i>Eukiefferiella</i> * (D)	Heptageniidae (E)	<i>Stictochironomus</i> * (D)
	Uenoidae (T)	<i>Chironomus</i> * (D)	Tricorythidae (E)	<i>Isoperla</i> (P)

E, Ephemeroptera; P, Plecoptera; T, Trichoptera; D, Diptera; C, Coleoptera; M, Megaloptera; Oligo, Oligochaeta; A, Amphipoda; I, Isopoda.

\*Significant genera within a family that is not significant.

either data set include altitude, catchment area,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ , ANC, pH, TN, TP, TSS, turbidity, DOC, specific conductance,  $\text{NH}_4^+$ , mean RPB habitat score, latitude and longitude (Table 5). At least one of the ordination axes for both taxonomic levels was significantly correlated ( $P < 0.0001$ ) to per cent EPT at both family and genus.

**Chi-square tables.** Table 4 lists the discriminating families and genera for each region. For both regions combined there were five families and 10–13 genera within the order Ephemeroptera, four to seven families and eight to 10 genera within the order Plecoptera, three to five families and five to seven genera within the order Trichoptera and two families and 15–32 genera within the order Diptera that were significant. All other orders or phyla had only one to two families and two to three genera that were significant. In general, most families that were significant had corresponding genera within that family that were

also significant. In the Plateau, two families (Hydroptychidae and Veliidae), but not their corresponding genera, discriminated, and 10 genera (all chironomids), but not their families, discriminated. In the Ridge and Valley, one family (Pleuroceridae), but not its component genera, and 43 genera, but not their families, discriminated. Twenty-eight of the 43 discriminating genera were chironomids.

## Discussion

The levels to which macroinvertebrates are commonly identified vary among state and federal agencies, academic researchers and taxonomic consulting firms. European researchers in general, identify macroinvertebrates to species with greater ease than researchers in the U.S. (because the macroinvertebrate fauna in European streams is better known). In our study of the MAH, one finding was particularly surprising: <30% of all specimens in the four major aquatic insect

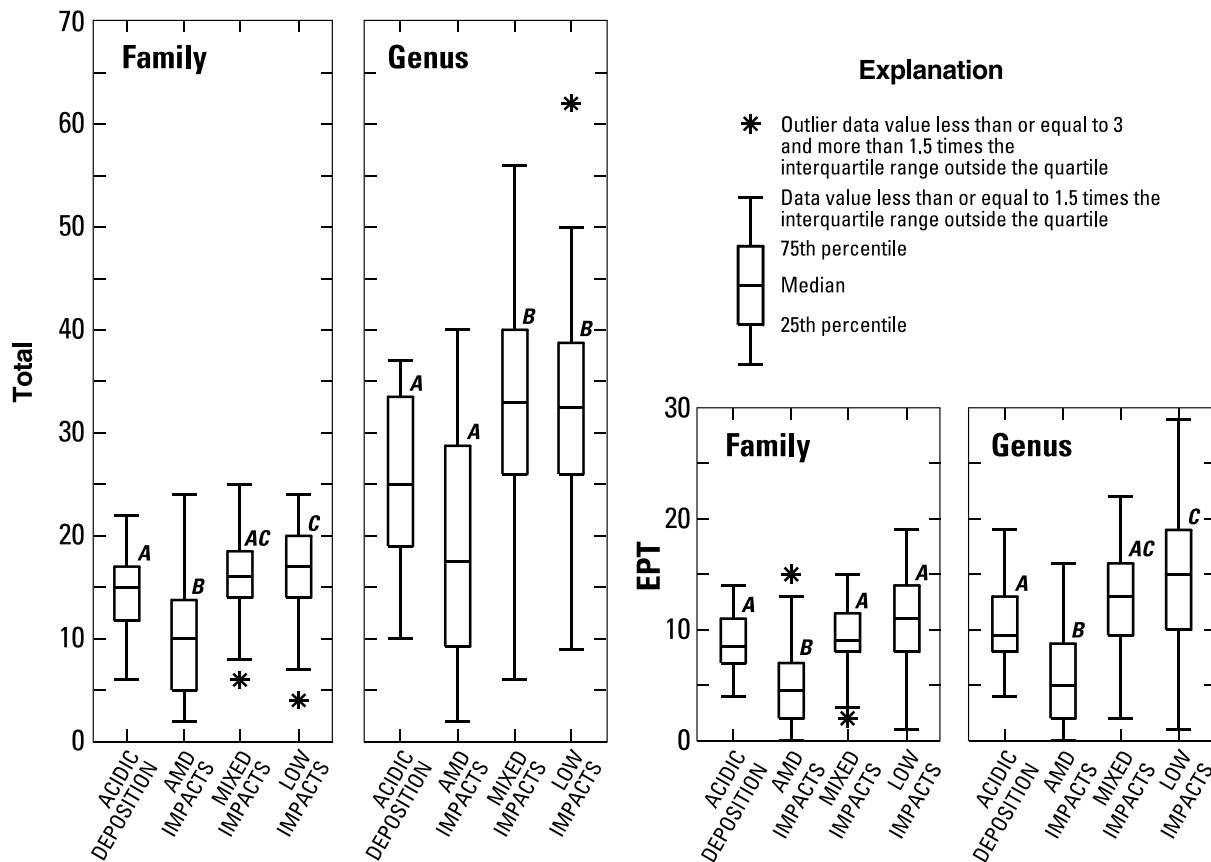


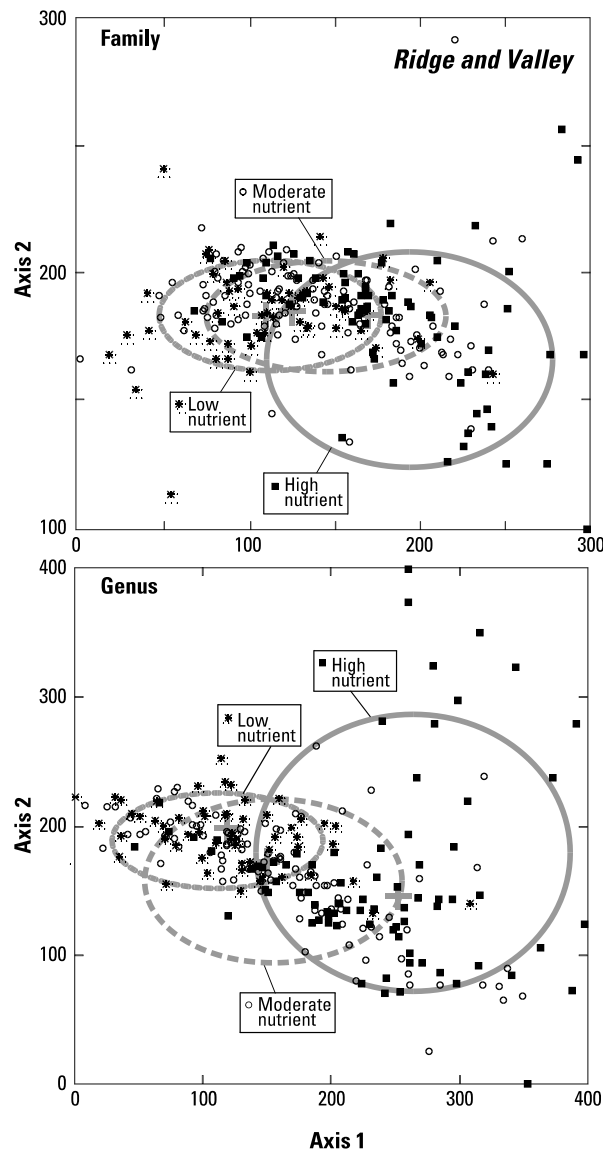
Fig. 3 Box plots for total taxa and EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa richness for family and genus identification level by impact class for the 239 sites in the Plateau region. Non-significant pairwise comparisons (Tukey's,  $P < 0.05$ ) within family or genus richness metrics are indicated by like letters.

orders (Ephemeroptera, Plecoptera, Trichoptera and Diptera) from the 490 sampling sites were identified to species. As a result, in this study we were not able to evaluate the effect of species taxonomy in bioassessments; we therefore focused on family versus genus analyses. The only reference to taxonomic level proportions we could find in the literature reported that 50% of the specimens from 165 reference sites were identified to species with 91% accuracy, whereas specimens were identified to at least family 98% of the time with 99% accuracy (Hewlett, 2000). Macro-invertebrate data sets that have a low proportion of specimens identified to species probably are a common situation, particularly in North America.

#### Relative discrimination power: family versus genus

Does finer taxonomic resolution of macroinvertebrates (for example classification to genus or species versus

family) allow better discrimination among sites differentially impacted by human activity? Bailey *et al.* (2001) focus on whether there is a difference in the amount of variation at the different taxonomic levels, with the idea that in a study area, more variation (i.e. more taxa with more ecological variation) within a level allows better possible ecological discrimination. They state that for the data they evaluated, 'there is as much or more variation among families within orders and orders within the Insecta as there was among genera within families'. Lenat & Resh (2001) calculated the ratios of the mean number of genera per family in North America for the orders Ephemeroptera, Plecoptera and Trichoptera, and the dipteran families Chironomidae and Simuliidae: they report integer ratios of four, 11, seven, 207 and 11, respectively, and species per family ratios were much higher. These same ratios calculated for the MAH study were in general about half the values calculated



**Fig. 4** The first two axes of the detrended correspondence analysis ordination plot of 251 sites at the family and genus level for the Ridge and Valley region, coded by impact class: high, moderate and low nutrient. Ellipses are drawn for each impact class based on the 90th and 10th percentile of the axis scores; the centroid for each ellipse is designated by a large +. Nine high nutrient sites on the family plot (>300 on either axis 1 or <100 on axis 2) and seven high nutrient sites (>400 on axis 1) on the genus plot are not shown so that the plots could be expanded for better visual clarity.

by Lenat & Resh (2001). For all orders except Diptera, ratios averaged three to four genera per family and were closer to the results from Bailey *et al.* (2001) stated above. Hawkins & Norris (2000) report genus to family ratios for EPT orders for data from Great

Britain, Australia and California that all are less than or equal to the ones from this study.

**Coarse impacts.** In the evaluation of coarse impacts, genus was only slightly better than family (high versus low impact classes) for both AD/MD and stream order in the Plateau, and the two taxonomic levels were similar in detecting the coarse nutrient impacts in the Ridge and Valley, though genus was significantly better in distinguishing among stream orders in this region (Table 3). Therefore, overall, family performed about as well as genus in detection of coarse impacts in both regions (except for stream size in the Ridge and Valley), supporting our general hypothesis. Ferraro & Cole (1995) and Wright *et al.* (1995) showed that family or higher levels, as well as finer taxonomic levels, were able to distinguish coarse impacts from sewage outfalls; however, sewage outfall pollution (point source) may be more severe than the effect of MD/AD impacts (non-point source) in our study. In contrast, Guerold (2000) found that family EPT diversity and abundance indices strongly underestimated the differences among acidic and reference streams compared with genus and species indices and stated that the use of family level identification would lead to erroneous interpretations.

**Subtle impacts.** Both family and genus were similar in their ability to detect differences in impact and size classes in the Plateau (Table 3), however, genus seemed to detect the natural subtle differences better than family and was slightly better in detecting the subtle human impacts in the Ridge and Valley region. Overall therefore, a coarser taxonomic level (family) was not able to detect the subtle differences as well as the finer taxonomic level (genus) though the distinction between family and genus was not dramatic. However, although we used four impact classes in the Plateau region, family was able to detect both coarse and subtle differences as well as genus though it often had lower significant values. In a study on California streams, Hawkins *et al.* (2000) found that family was not able to distinguish subtle impacts as well as species did. They found that RIVPACS-type species models were able to detect relatively subtle non-point source impacts from logging that family models could not, however, they were comparing species models to family based models, a larger difference in taxonomic resolution than we were able to evaluate.

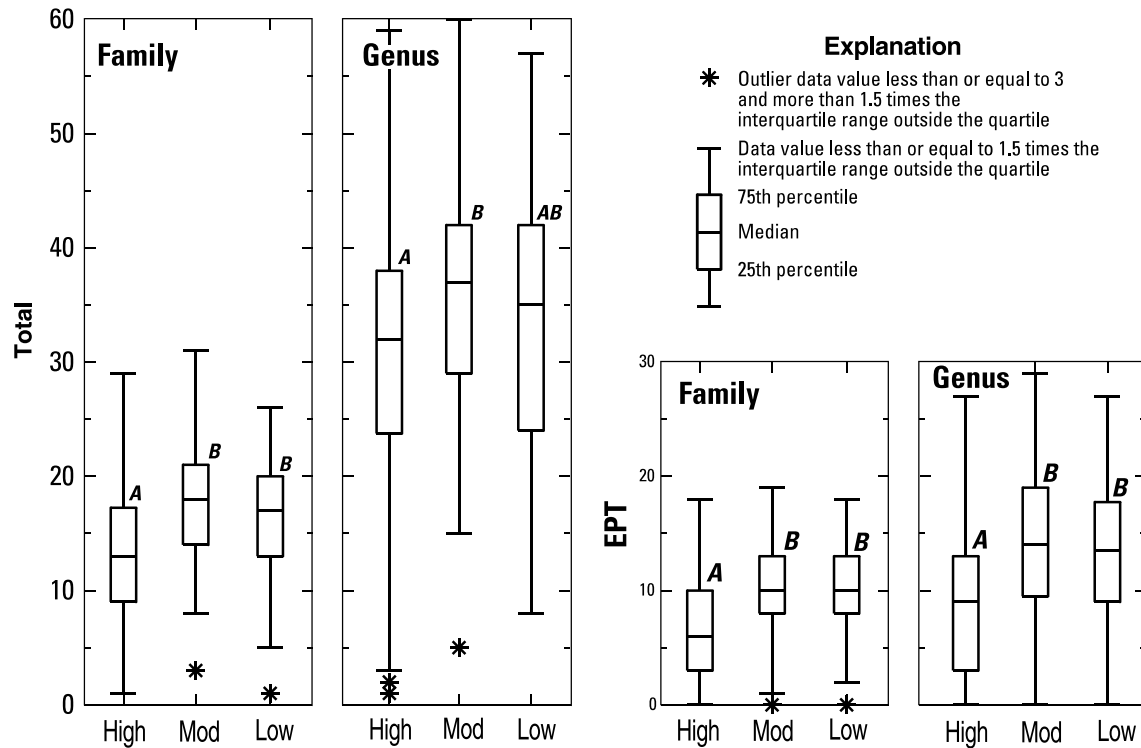


Fig. 5 Box plots for total taxa and EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa richness for family and genus identification level by impact class for the 251 sites in the Ridge and Valley region. Non-significant pairwise comparisons (Tukey's,  $P < 0.05$ ) within family or genus richness metrics are indicated by like letters.

Overall, the differences in discrimination within the Plateau and Ridge and Valley regions are probably because of a combination of impact and stream gradient differences. The AMD and AD impacts in the Plateau can have direct toxicity on the benthic organisms as well as the frequent habitat impacts in AMD affected streams, therefore there is the potential for many taxon specific responses (i.e. high acid and metal toxicity for Ephemeroptera versus relative tolerance by Plecoptera). However, impacts because of nutrients (Ridge and Valley) are primarily indirect and therefore lower potential for taxon specific responses (although increased nutrients because of agricultural land-use are often accompanied by increased sedimentation and habitat affects which can have direct consequences). The Ridge and Valley also had a higher range in stream gradient within the three stream orders than the Plateau streams and nutrients and sediments were higher in the valley streams (primarily third order) than in the ridge streams (primarily first order), in other words, the gradient in nutrient and sediments followed a stream size/slope gradient.

#### Natural and disturbance gradients

Determination of impact classes is often not the only goal of bioassessment. Researchers also often want to relate biological responses to environmental gradients to help identify hypotheses related to causes of degradation or to identify important natural gradients. Does taxonomic level affect relationships between biota and environmental gradients such as gradients in water chemistry, habitat and land use (Furse *et al.*, 1984; Wright *et al.*, 1984; Bowman & Bailey, 1997)? Using a variety of multivariate analyses both family and genus showed most of the same correlations to environmental variables with minor exceptions for the two regions. Our results are consistent with those of Furse *et al.* (1984), Marchant *et al.* (1995) and Bowman & Bailey (1997): genus used in multivariate analysis often results in more precision or statistical power in discrimination among sites than family; nevertheless, family usually provides the same basic distinction among sites and identifies the same relationships to environmental gradients. Although genus or species may only provide a slight

**Table 5** Spearman rank correlation coefficients and significance of variables correlated to axis 1 and 2 of family and genus ordinations for the Plateau and Ridge and Valley regions

Variables	Family		Genus	
	Axis 1	Axis 2	Axis 1	Axis 2
<b>Plateau</b>				
ANC	-0.26***	0.18**	0.41***	-0.02
SO <sub>4</sub> <sup>2-</sup>	0.03	0.24***	0.19**	0.24***
Cl <sup>-</sup>	-0.16*	0.34***	0.30***	0.16*
TP	-0.03	0.26***	0.15*	0.18**
TN	0.02	0.31***	0.19**	0.26***
TSS	0.16*	0.10	-0.13*	0.17**
RBP Habitat	-0.03	-0.19**	-0.16*	-0.24***
Altitude	0.33***	-0.06	-0.29***	0.16*
Catchment area	-0.49***	0.17*	0.66***	-0.05
<b>Ridge and Valley</b>				
TP	0.41***	-0.04	0.49***	-0.37***
TN	0.46***	0.04	0.59***	-0.36***
TSS	0.29***	-0.02	0.39***	-0.30***
ANC	0.52***	0.10	0.67***	-0.43***
SO <sub>4</sub> <sup>2-</sup>	0.16**	-0.08	0.25***	-0.01
Cl <sup>-</sup>	0.52***	-0.06	0.62***	-0.31***
RBP Habitat	-0.40***	0.20**	-0.43***	0.23***
Altitude	-0.26***	0.20**	-0.27***	0.13*
Catchment area	0.13*	0.19**	0.25***	0.03

ANC, acid neutralising capacity; TP, total phosphorus; TN, total nitrogen; TSS, total suspended solids.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

improvement over family, they often provide substantially greater number of taxa than family and thus more lines of specific ecological evidence that we believe may be vital in the interpretation of the whys and so what's of bioassessment and gradient research. These conclusions are corroborated by Guerold (2000) who states that taxonomic level depends on the objectives: 'if the purpose of a study is simply to detect an impact of a perturbation on macroinvertebrate communities, determination to family-level may be used, but ecological interpretation remains hazardous'.

It is possible that most of the improved performance of genus versus family in our study was caused by the added information of chironomid genera (large number of chironomid genera in family versus few genera per family for all other families). We found many chironomid genera to be significant in distinguishing among impact classes (chi-square, Table 4). King & Richardson (2002) found that chironomids at genus level, but preferably at species level, were important in bioassessments of impact zones in wetlands. They stated that species-level chironomid

data combined with family-level data for all other taxa yielded the same wetland bioassessment as identifying all taxa to genus or species (King & Richardson, 2002). Hawkins & Norris (2000) also considered the chironomids to be an important group; however, exclusion of this group in their analyses did not change their ability to detect group classification in a species model. They suggest that as they had a diverse fauna identified to species, the information attributed to the chironomids was possibly redundant. Rabeni & Wang (2001) assessing the ability of only four metrics to distinguish 13 reference sites from 13 agriculturally influenced sites from two ecoregions in Missouri (primarily organic pollution) found that metrics calculated without chironomids distinguished these two groups better than metrics that included the chironomid data. Their results might have been sensitive to the use of only four metrics; the use of some of these has been strongly criticised (e.g. Shannon's diversity index, Hurlbert, 1971). Also their number of chironomid taxa (25–35) was substantially lower than the number in our study. Both factors might account for their observation that inclusion of chironomids lowered their sensitivity. The chi-square results from our study, however, illustrate the potential importance of chironomid genera compared with other taxa (Table 4): for both regions combined, EPT orders individually only had three to seven families and five to 13 genera that were significant, yet although there were only one to two significant Dipteran families, there were 15–32 significant genera and 67 and 88% of these were chironomids in the Plateau and Ridge and Valley regions, respectively. Therefore, unlike in the Hawkins *et al.* (2000) study, the chironomids in this study added important information that was not redundant, probably because most of the MAH insects were only identified to genus, whereas Hawkins *et al.* (2000) had species data. We believe that bioassessment research and monitoring will be able to better distinguish among impact groups in general and be more statistically robust if chironomids are identified to at least genus level.

Bailey *et al.* (2001) recommend that when conducting general biomonitoring of new sites, investigators should first record macroinvertebrate assemblage structure and composition at the family level based on a subsample of 200–500 organisms. In their opinion, this should provide sufficient detail to allow multivariate analysis or index calculations necessary

to evaluate bioassessment relative to other sites and appropriate reference condition. They also suggest that additional information should be gathered to complement the above data by collecting data on a short list of critical indicator taxa pertinent to the particular study or region. A larger portion of the sample would be processed specifically to find all of these critical indicator taxa so that there would be little chance of missing an indicator taxon even if the taxon were quite rare. Based on the results from our study, Bailey et al.'s recommendation to use a subsample identified to family along with detailed counts of indicator taxa seems appropriate if resources are limited. We suggest family for all taxa except the chironomids, our indicator group, which would be classified to genus or species. This combination of taxonomic levels was also shown to work well by King & Richardson (2002) for wetland bioassessments. However, additional counts as suggested by Bailey et al. (2001) may not be needed. In our study significant information was added by identifying chironomids to genus without increasing the number of specimens identified compared with all other insect groups (i.e. fixed count subsampling).

Finally, if a combination of taxonomic levels is considered, such as all taxa to family except the chironomids to genus or species, there may not be a significant cost savings compared with identifying all taxa to the finer level. But a final caveat comes into play; if comparative analyses are to be done with other agency studies, genus data can always be summed back to a higher level such as family if needed, but the opposite is not an option. In addition, as our knowledge of ecological requirements of macroinvertebrates improves and better taxonomy adds more genera and species within families, thus more information and less single-genus families, there may be even more reasons in the future to wish that all past data were at the genus/species level, particularly when assessing temporal trends or combining data across studies to determine distributions and ecological requirements. Therefore, even though there was only a slight improvement in detection of natural and impact classes in going from family to genus level data in our study, as taxonomy and our ecological understanding improves, this difference should only get greater. Cranston (1990) states 'it is fundamental that species be recognised first and their phylogenetic relationship assessed before attempts are made to

introduce rigor into the reduction of the level of taxonomic determination'.

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